Phylogenetic analysis and mutation analysis of multi species Laccases

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ABSTRACT

Laccase (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) plays very important role in the environmental applications. Laccases are the largest subgroup of multicopper oxidases having the capacity to oxidize phenol compounds. The first laccase was identified in plant species in Japanese lacquer tree *Rhus vernicifera*. Later this enzyme was also isolated from the other diverse species like bacteria, fungi, insects etc. The majority of laccases have been identified from higher fungi, mostly in white rot fungus. But much importance has been given to bacterial laccase because of its ease of culture and maintenance. Laccase is synthesis in both extracellular and intracellular organisms. In this study, a phylogenetic analysis with 10 species of plants, 13 species of bacteria, 28 species of fungi, 09 species of insects which are the representatives of laccase producers were analysed. Various phylogenetic algorithms were used to construct trees for these organisms and fungi was choose as the outgroup, since they are considered as the most primitive of the lot. Manual multiple sequence alignment was performed using Bioedit ver. 7.1.11. Mutation and divergence rates of the laccases were calculated. PHYLIP 3.7 was used to perform the phylogenetic analysis.

KEY WORDS: Laccase, multiple alignment, BIOEDIT, PHYLIP, Mutation and divergence rate

1. INTRODUCTION

Laccases are important for many reasons, especially for their environmental applications. Laccase (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are the largest subgroup of multicopper oxidases having the capacity to oxidize phenol compounds. Laccase oxidize polyphenols, methoxy-substituted phenols, diamines and other compounds, but it do not oxidize tyrosine.

Phylogenetics is the study of evolutionary relationships. Phylogenetic analysis is the means of inferring or estimating these relationships. The evolutionary history inferred from phylogenetic analysis is usually depicted as branching, treelike diagrams that represent an estimated pedigree of the inherited relationships among molecules (‘‘gene trees’’), organisms, or both. (Fiona Brinkman and Detlef Leipe, 2001)

Maximum parsimony (MP) is an optimization criterion that adheres to the principle that the best explanation of the data is the simplest, which in turn is the one requiring the fewest ad hoc assumptions. In practical terms, the MP tree is the shortest—the one with the fewest changes—which, by definition, is also the one with the fewest parallel changes. There are several variants of MP that differ with regard to the permitted directionality of character state change (Swofford et al., 1996).

2. MATERIALS AND METHODS

2.1. Extraction of Sequence from database: Complete gene sequences from 10 species of plants, 13 species of bacteria, 28 species of fungi, 09 species of insects which synthesise laccase were obtained from the the NCBI database.

Among bacteria the laccase synthesising organisms ones selected were *Azospirillum lipoferum, Bacillus subtilis, E.coli, Marinomonas mediterranea, Pseudomonas aeruginosa, Pseudomonas syringae, Streptomyces cyaneus, S.ipomoea, Stenotrophomonas maltophilia, Stereum ostrea, S.hirsutum, S.lavandulae, Thelephora terrestris*. Among plants the laccase synthesisers chosen were *Acer pseudoplatanus, Aesculus parviflora, Lentinula edodes, Mangifera indica, Pinus mugo, Pinus taeda, Pistachio Palestine, Prunus domestica, Schinus molle, Zinnia elegans* of the fungi the laccase synthesisers chosen were - *Armillaria mellea, Aspergillus nidulans, Aspergillus niger, Ceriporiopsis subvermispora, Cerrena unicolor, Coprinus cinereus, Coriolopsis byrsina, Coriolus hirsutus, Cyathus olla, Diaporhte phaseolorim, Fomitella fraxinea, Ganoderma lucidum, Heterobasidion annosus, Lentinus tigrinus, Lenzites betulinus, Pestalotiopsis uvicola, Phanerochaete chrysossporium, Phlebia radiate, Pleurotus ostreatus, Pycnoporus cinnabarinus, Pycnoporus sangunieus, Trametes hirsutus, Trametes ochracea, Trametes trogii, Trametes versicolor, Trametes villosa, Trichoderma atoviride, Trichoderma longibrachiatum*.

The insects synthesizing laccases chosen were *Bombyx mori, Dorsophila melanogaster, Manduca sexta, Megacopta punctatissima, Musca domestica, Nysius plebeius, Papilio palytes, Riptortus pedestris, Tribolium castaneum.* All the sequences were retrieved National Centre for Biotechnology Information (NCBI). Bioedit sequence alignment editor copyright (c) 1997-2011 was used to perform the multiple sequence alignment and the sequence was then manually edited. The fungal species were used as the outgroup as they are considered the most primitive laccases.

2.2. Construction of a Phylogenetic Tree: The genotypes were transformed into 0 and 1 characters by using FACTOR program from PHYLIP package version 3.57c. These data were collected and used next in the MIX program also from PHYLIP with which the evolutionary relationships were estimated based in the Wagner parsimony method. The significance of the branching in the Wagner network was done by bootstrapping. We used the program SEQBOOT of the PHYLIP package to perform 1,000 bootstraps. The number of times that a given branching was observed was used to evaluate the robustness of the tree.

2.3. Steps involved in the DNAPARS maximum parsimony analysis: The sequence file was converted into the NBRF/ PIR format using Readseq. BIOEDIT was used to perform the multiple alignment with a .PHY output – gave first line with numbers of sequences and length of alignment, and then sequences interleaved. *.PHY file was renamed as "infile". Seqboot.exe was run with 1000 replicates option which gave an outfile. The outfile was renamed as the "infile". DNApars.exe was run using multiple (20) data sets option which gave multiple treefiles. Treefile was renamed as the "intree". Consense.exe was run which gave a consensus treefile. The rooted tree plotting program Drawgram.exe was run and the tree was previewed using the MS Windows display option. The tree was plotted, renaming and saving it with *.jpeg extension. Treeview was run on the "treefile" – and the bootstrap values at the internal edge labels were obtained. The tree was imported and the phylip tree edited with the corresponding bootstrap values. The outtree file was opened with Treeview program to evaluate the topologies of various tree types including radial, clad and phylogenic trees. The entire protocol is summarized in Figure 1.

3. RESULT AND DISCUSSION

The sequences were extracted from NCBI and using Bioedit tool, manual MSA (multiple sequence alignment) was preformed (Figure-2). While performing MSA, only the concenses sequences were taken and the other unaligned sequences were deleted. This file is saved in .phylip format and the Phylogenetic Analysis of sequences of all the taxa was done using PHYLIP (Figure-3). From the tree, 5 Operational Taxonomic Units (OTUs) four from bacterias *Streptomyces ipomoea* (B8), *Stereum ostrea*(B10), *S.lavandulae*(B12), *Thelephora terrestris*(B13) will fall in one group with the fungus *Aspergillus nidulans*(F2) *as an* outgroup and the others taxas coming in a single group (Figure-4).

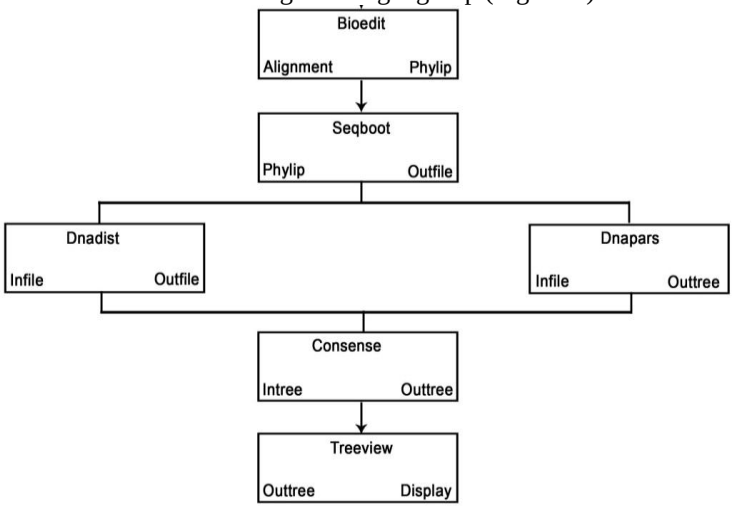

Figure 1. Schematic presentation of the protocol used.
***Top entries – software tool;*Bottom entries – input and output types**

Figure 2. The aligned sequences of all the taxa

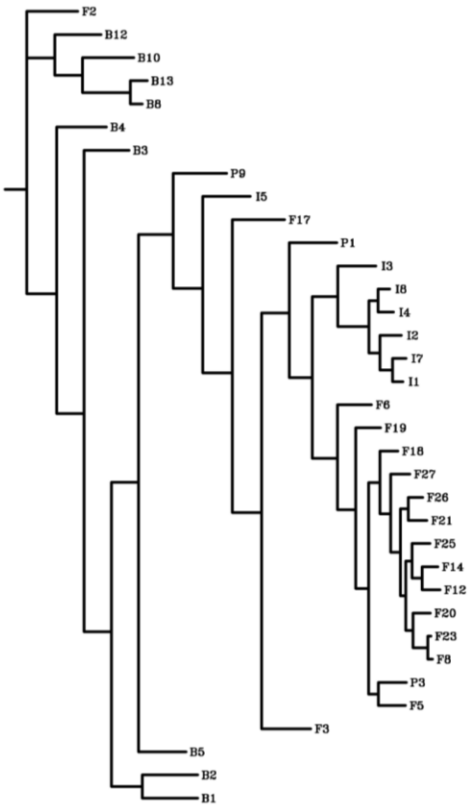

Figure.3.The Phylogenetic Analysis of sequences of all the taxa

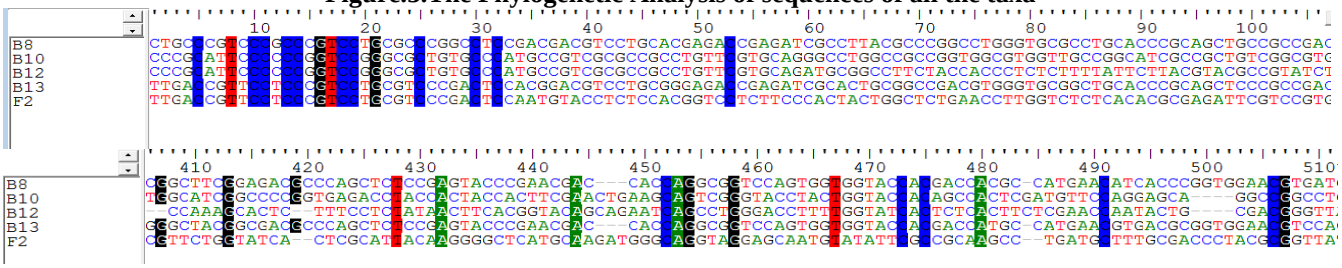

Figure.4.The aligned sequences of 5 OTUs

4. CONCLUSION

In conclusion, the results of this preliminary study indicate that Maximum Parsimony appears to be a very reliable method for phylogenetic inference among all the taxa using gene sequences. In the phylip tree, we have a 5 OTUs B8, B10, B12, B13 clustering together and all the remaining 53 OTUs clustered into a single group. The reason for this appeared to be mutations that occurred in the bases 8 to 55 and 390 to 500. The correlation of the tree topology and the multiple sequence alignment led us to identify this finding. Further study is undertaken to characterized the changes in the conformation of the laccase enzyme which probably might also imply the variations that exist among the laccases of individual species.

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